IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Shuji TERASHIMA et al.
Serial No. 10/575,099 Group Art Unit: 1633
Filed: October 8, 2004
Title: METHOD FOR PREPARING CELL CONCENTRATE AND CELL COMPOSITION

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner for patents,
P. O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

I, Mikitomo YASUTAKE, declare:
That I am a citizen of Japan; and my full post office address is 147-405, Bentencho, Shinjukuku, Tokyo, Japan:

That my education, employment and research history are as follows:

Education:

March 1987, Graduation from Biology Department of the Faculty of Science of Kyushu University

Employment:

April 1987: Asahi Chemical Industry Co., Ltd

October 1995: New business development division of Asahi Medical Co., Ltd.

June 2000: Chief Researcher of Development Laboratory of Asahi Medical Co., Ltd.

April 2005: Vice General manager of Technology Development Depertment of Functional Product Division of Asahi Kasei Medical Co.,Ltd.

July 2007: General Manager of Research and Development Department of Medical Product Development Division of Asahi Kasei Kuraray Medical CO.,LTD.

Research career:

October 1995: Research scholar at Cancer Pathology Division of the Institute of Medical Science, the University of Tokyo

September 1998: Research scholar at Cell Processing Study Division of the Institute of Medical Science, the University of Tokyo

September 2006: Received Doctor of Medicine from the University of Tokyo, Visiting Researcher at Cell Processing Division of the Institute of Medical Science, the University of Tokyo

July 2008: MIL researcher at Institute of Advanced BioMedical Engineering and Science, Tokyo Women's Medical University

I declare further that the following statements are true and correct to the best of my knowledge.

I believe that the present invention is not obvious from Sumita et al. (US Patent 6,268,119) in view of Oka et al. (US Patent 5,298,165), Oka et al. (US Patent Application Publication 2004/0251195), Fukuda et al. (WO02/087660) and Rubinstein et al. Proc Natl Acad Sci USA, 1995, 92:10119-10112. The reasons are mentioned below.

Claims 1, 6 and 12-17 of Sumita et al. (US Patent 6,268,119) are as follows.

1. A cell separation method comprising the steps of:

(a) introducing a cell-containing fluid, containing cells to be recovered and cells to be removed, into a cell-capturing means which substantially captures said cells to be recovered and substantially permits passage therethrough of said cells to be removed, whereby said cell-capturing means comprises one of a porous structure of nonwoven fabric having a fiber diameter of 1.0-30 μm and a porous spongy structure having a pore size of 2.0-25 μm;

(b) taking out the resulting fluid containing the cells to be removed from said cell-capturing means; and
(c) introducing a liquid with a viscosity of not more than 100 mPa.multidot.s and not less than 5 mPa.multidot.s into said cell-capturing means at a linear speed of at least 0.5 cm/min to recover therefrom said cells to be recovered which have been captured by said cell-capturing means.

6. A cell separation method according to claim 1, wherein the cell-capturing means is obtained by packing said porous structure into a container having a liquid inlet and a liquid outlet.

12. A method according to claim 1, wherein the direction of introduction of the liquid with a viscosity of not more than 500 mPa.multidot.s and not less than 5 mPa.multidot.s is opposite to the direction of introduction of the cell-containing fluid containing cells to be recovered and cells to be removed.

13. A cell separation method according to claim 1, wherein the cells to be recovered are nucleated cells.

14. A cell separation method according to claim 13, wherein the nucleated cells are a mononuclear cell fraction containing hematopoietic stem cells.

15. A cell separation method according to claim 13, wherein the nucleated cells are hematopoietic stem cells.

16. A cell separation method according to claim 1, wherein the cells to be removed are cells having no nucleus.

17. A cell separation method according to claim 16, wherein the cells having no nucleus are at least one of erythrocytes and platelets.

The aforementioned claims do not recite the following essential feature of claim 1 of the present application: "wherein the above-described method is characterized in that the cell-containing solution that contains nucleated cells and unnecessary cells are separated into a layer that is rich in nucleated cells and a layer that is rich in unnecessary cells, the layer rich in unnecessary cells is first introduced into the

above-described filter device, and the layer rich in nucleated cells is then introduced therein, so as to discharge the unnecessary cells remaining in the above-described filter device while capturing the nucleated cells by the above-described filter material,"

Similarly, the aforementioned claims do not recite the following essential feature of claim 12 of the present application: "wherein the above-described method is characterized in that it uses a filter device formed by packing a container having an inlet and an outlet for a cell-containing solution with a filter material obtained by stacking a nucleated cell-capturing material and a recovery solution-rectifying material, which consist of porous bodies wherein the value obtained by dividing the effective filtration area of the above-described filter material by the thickness of the nucleated cell-capturing material packed is between 15 and 120 cm, such that a nucleated cell-capturing material is located on the inlet side of a cell-containing solution,".

The object of Oka et al. (US Patent 5,298,165) is to provide a method for removing leucocyte by which the remaining rate of leucocyte in the leucocyte-containing blood preparation is 10^{-4} or less (column7, lines22-25). The advantageous effect which is described in Oka et al. (US Patent 5,298,165) is that pressure loss at filtering is small, that great decrease of flow rate due to treatment of blood preparation does not occur, and that the remaining rate of leucocyte is 10^{-4} or less. (column21 "INDUSTRIAL APPLICABILITY"). Oka et al. (US Patent 5,298,165) discloses nothing about a method for recovering captured leucocyte, and about the recovery efficacy of leucocyte. The description that "the remaining rate of leucocyte is low, namely the capture rate of leucocyte is high" does not suggest that the captured nucleated cell can be efficiently recovered." Rather, since high capture rate of leucocytes implies strong adsorption of leucocytes to non-woven fabric, one skilled in the art would expect that the recovery rate of leucocytes is decreased, and would not try to use the filter of Oka et al. (US Patent 5,298,165) for recovering the nucleated cells.

Oka et al. (US Patent Application Publication 2004/0251195) discloses that leucocytes are filtered out after blood samples are separated into several blood components by centrifugation (paragraph 0005). However, Oka et al. (US Patent Application Publication 2004/0251195) discloses nothing about a method for preparing a cell concentrate wherein the layer rich in unnecessary cells is first introduced into the filter device, and the layer rich in nucleated cells is then introduced therein, so as to recover the nucleated cells as recited in claim 1 of the present application. Oka

(US2004/0251195A1) relates to a blood processing filter for removing leucocyte. Oka discloses generally that whole blood is separated into several blood components by centrifugation, and then removal of leukocytes is carried out. Oka (US2004/0251195A1) does not disclose the feature and advantageous effect of the present invention that the layer rich in unnecessary cells is first introduced into the filter device, and the layer rich in nucleated cells is then introduced therein, so that the nucleated cells and the unnecessary cells are efficiently separated from each other.

Fukuda et al. (WO02/087660) discloses a method of removing leucocytes from blood samples by forming a blood cell concentration gradient in pooling unit before introducing blood into a filter for eliminating leucocytes (Abstract). In Fukuda et al (WO02/87660), before introduction of blood into leukocyte removal filter, blood concentration gradient is formed in a blood pooling unit, and the blood is filtered. Thus, leukocyte can be efficiently removed and platelet can be recovered at high recovery rate. An object of the invention of Fukuda et al. (WO02/087660) is to improve the filtration performance of the filter, and provide a method for filtration of blood which can be easily operated and an automated filtration device suitable for said method. Fukuda et al. (WO02/087660) describes as an advantageous effect that leucocytes can be efficiently removed, and platelets can be recovered highly. Fukuda et al. (WO02/087660) does not disclose a method for recovering the nucleated cells captured by the filter material, and does not disclose than the nucleated cells can be efficiently recovered. Fukuda et al. (WO02/087660) describes that leucocytes can be efficiently removed, but this description does not suggest that the nucleated cells can be efficiently recovered. Rather, one skilled in the art would expect that the recovery rate of the leucocytes is decreased, since efficient removal of leucocytes implies strong adsorption of leucocytes to non-woven fabric.

The present invention is characterized in that in the method for recovering nucleated cells, the recovery rate of nucleated cells is increased by previously separating a cell-containing solution into a a layer that is rich in nucleated cells and a layer that is rich in unnecessary cells. On the other hand, Fukuda et al is characterized in that in a method for removing leukocyte, the removal ratio of leukocyte is increased by forming blood cell concentration gradient in blood which contains leukocyte. In Fukuda et al, lowest layer containing erythrocyte is first flowed, and then a second layer containing leukocyte is flown, as a result of specific gravity. However, Fukuda et al relate to a method of removing leukocyte. In order to increase the removal ratio of leukocyte in

Fukuda et al, leukocyte which is to be removed must be introduced into the filter material before plasma proteins (such as albumin) which suppress blood adherence are introduced into the filter material (page 10 of the specification, WO02/087660). The lowest layer containing erythrocyte, and the second layer containing leukocyte are not introduced into the filter separately, but are introduced into the filter together with each other before the third layer of plasma containing platelet.

Fukuda et al disclose only a technical concept that erythrocyte and leukocyte which are to be removed are first introduced together with each other, and then plasma which contains platelet is introduced. Fukuda et al neither teach nor suggest any technical concept that a lowest layer containing erythrocyte and a second layer containing leukocyte are introduced into the filter separately so that the nucleated cells (such as leukocyte) of the second layer is recovered at high yield.

As mentioned above, both Oka et al. (US Patent Application Publication 2004/0251195) and Fukuda et al. (WO02/087660) relate to a system for removing cells (namely, leukocyte), while the present invention relates a system for recovering cells. In Fukuda, the blood cells are separated from the heavy cell to light cell by specific gravity (namely, erythrocyte → granulocyte/monocyte → lymphocyte → platelet/plasma), and these cells are introduced into the leukocyte removal filter in this order. However, the object and effect of the Fukuda (namely, leukocyte is efficiently removed, and high recovery rate of platelet is achieved.) is different from those of the present invention. Therefore, there is no motivation to combine "blood concentration gradient for removing cells" of Fukuda with Sumita et al. Therefore, I believe that the present invention is not obvious from Sumita et al and Fukuda et al.

The feature of the present invention is resides in that nucleated cells and unnecessary cells are separated into a layer that is rich in nucleated cells and a layer that is rich in unnecessary cells, the layer rich in unnecessary cells is first introduced into the filter device, and the layer rich in nucleated cells is then introduced therein". The unnecessary cells are first introduced into the filter device, and the nucleated cells are then introduced therein later. Thus, many of nucleated cells stay at the upper part of the filter, and nucleated cells can be recovered more certainly from the filter by reverse flashing by recovering fluid. Further, by introducing the nucleated cells later, an advantageous effect is obtained that unnecessary cells which have stayed inside the

filter are washed out. Namely, the layer rich in nucleated cells have a function of holding the nucleated cells at upper part as well as a function of washing out the unnecessary cells (=a function as a washing solution). In the present invention, by the aforementioned feature, a method for preparing a cell concentrate can be realized wherein the risk of contamination is reduced and the recovery rate is high. The following advantages can be achieved in the method of the present invention.

(1) The unnecessary cells remaining on the filter can be washed with the layer that is rich in nucleated cells. Thus, the amount of the unnecessary cells remaining on the filter can be decreased, and the purity of the recovered nucleated cells is increased.

(2) As compared with the case where an un-separated cell-containing solution is introduced into the filter device, the amount of liquid which is used for capturing the nucleated cells on the filter material can be decreased. The nucleated cells remains near the surface of the filter material, and thus the nucleated cell can be easily recovered.

As demonstrated by the comparison between Examples 1 and 2 and Comparative example 1 of the present application, the volume of erythrocytes can be reduced and mononuclear cell recovery rate can be increased by the operation of first introducing the layer rich in unnecessary cells into the filter and then introducing the layer rich in nucleated cells into the filter" as recited in the claim of the present application. The aforementioned advantageous effects of the present invention cannot be expected from Sumita et al, Fukuda (WO02/87660) and Oka. Therefore, the present invention shows advantageous effects which cannot be expected from the cited references.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Dated of Feb. 3, 2011

Mikitomo YASUTAKE, Ph. D.